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Spotlight on Renin : Renin, Prorenin and the Putative (Pro)renin Receptor

AH Jan Danser and Jaap Deinum

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Spotlight on Renin

Renin, Prorenin and the Putative (Pro)renin Receptor

AH Jan Danser and *Jaap Deinum (*Department of Pharmacology, Erasmus MC, Rotterdam, The Netherlands and *Department of Internal Medicine, UMCN St. Radboud, Nijmegen, The Netherlands*)

Renin is an aspartic protease that consists of two homologous lobes. The cleft in between contains the active site with two catalytic aspartic residues. Renin cleaves angiotensinogen, to generate angiotensin I (Ang I). Ang I is the precursor of the active end-product of the renin-angiotensin system (RAS), angiotensin II (Ang II).

Renin has also been called active renin to underline that an enzymatically inactive form of renin exists: prorenin. Only with the cloning of the renin gene in 1984, was prorenin definitively proved to be the precursor of renin.¹ For reasons that are unknown, prorenin circulates in human plasma in excess to renin, sometimes at concentrations that are 100 times higher.²

A 43-amino acid N-terminal propeptide explains the absence of enzymatic activity of prorenin. This propeptide covers the enzymatic cleft and obstructs access of angiotensinogen to the active site of renin.

We provide here a synopsis of a recently published paper on renin/prorenin and the renin receptor.³

Prorenin activation

Prorenin can be activated in two ways: proteolytic or non-proteolytic. Proteolytic activation involves actual removal of the propeptide. *In vivo*, proteolytic activation of prorenin occurs in the kidney. Bolus infusions of recombinant human prorenin in monkeys did not provide evidence for prorenin-renin conversion in the circulation.⁴

Non-proteolytic activation of prorenin is a reversible process. It can best be imagined as an unfolding of the propeptide from the enzymatic

cleft (Figure 1). This unfolding consists of at least two steps. In the first step the propeptide moves out of the enzymatic cleft, and in the second step the renin part of the molecule assumes its enzymatically active conformation.⁵ Non-proteolytic activation can be induced by exposure to low pH (with an optimum at pH 3.3) and cold, called acid activation and cryoactivation, respectively.^{6,7} Acid activation leads to complete activity of prorenin, cryoactivation to partial (~15%) activity.

Non-proteolytically activated prorenin is enzymatically fully active, and can be recognised by monoclonal antibodies that are specific for the active site. Remarkably, these antibodies also recognised prorenin following incubation with a renin inhibitor.⁵ Application of monoclonal antibodies against the propeptide after prorenin exposure to a renin inhibitor confirmed that, under these conditions, the prosegment was still present.⁸ Thus, renin inhibitors, like low pH and cold, are capable of non-proteolytically 'activating' prorenin, although of course, due to the presence of the renin inhibitor, this activated prorenin cannot display enzymatic activity (Figure 1).

Measurement of renin and prorenin

Two types of assays exist for measurement of renin. The first one employs the enzymatic activity of renin: the plasma renin activity (PRA) assay. It is performed by incubating plasma in the presence of inhibitors of Ang I-degrading enzymes. The generated Ang I is an index of renin activity. This generation depends not only on the amount of renin but also on the angiotensinogen concentration in plasma.

The second type of renin assay is a direct immunoassay. Three assays are currently marketed, an immunoradiometric assay (IRMA) by Cis Bio,⁹ and two assays by Nichols Diagnostics,^{10,11} an IRMA and a chemoluminometric assay that runs on an automated platform. All three assays use an immobilised capture antibody that binds both renin and prorenin. The second, developing antibody is specific for renin and is labelled by either radioactive iodine for the IRMA or acridinium for the chemoluminometric assay.

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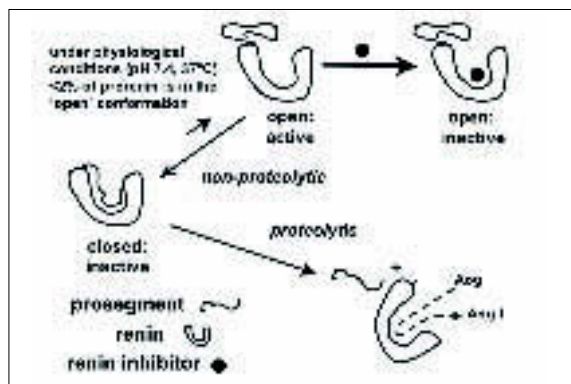


Figure 1 Proteolytic and non-proteolytic activation of prorenin. A renin inhibitor will increase the amount of non-proteolytically activated prorenin. Such a drug binds to prorenin when it is in its open, active conformation. Once bound, the prosegment cannot regain its original 'closed' position, and thus prorenin will now be recognised by antibodies directed against the active site, although of course it is incapable of generating angiotensin (Ang) I from angiotensinogen (Aog). Due to the high affinity of the renin inhibitor, prorenin will stay in the 'open' conformation, and thus the equilibrium will shift into the direction of the open conformation. Eventually, all prorenin will be in the open conformation. Reproduced with permission from reference 3.

All renin assays may overestimate renin because of the presence of cryoactivated prorenin. Samples should therefore never be left on ice for prolonged periods of time.

Prorenin can be measured indirectly by performing a renin assay after converting prorenin to renin (proteolytic or non-proteolytic).^{5,8} The results of this assay will reflect total renin levels, i.e. the levels of prorenin plus renin. Subtracting the renin level from the total renin level is then a measure of prorenin.

Tissue renin

Local synthesis of angiotensins at tissue sites, independent of angiotensin generation in the circulation, is now widely accepted.^{12,13} Interference with such locally synthesised Ang II may in fact underlie the beneficial effects of RAS blockers. For a long time it was thought that local angiotensin synthesis, e.g. in the heart, depended on locally synthesised renin. Although renin is indeed present in cardiac tissue,^{14,15} there is no evidence for its local synthesis in the heart. Thus, it appears that the renin required for cardiac angiotensin generation is taken up from the circulation, i.e., is kidney-derived. A similar concept applies to the vascular wall.¹⁶

An attractive concept is that, in tissues not synthesising renin locally, circulating prorenin, following its local activation, contributes to angiotensin generation. This would not only provide a role for prorenin *in vivo*, but also explain why tissues, in contrast to plasma, contain predominantly renin.¹⁴ In support of this concept, transgenic rodents with (inducible) prorenin expression in the liver display increased cardiac Ang I levels, cardiac hypertrophy and/or vascular damage.^{17,18}

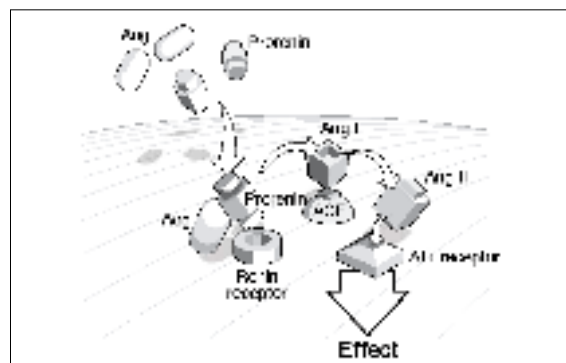


Figure 2 Cell surface angiotensin (Ang) generation by prorenin is a highly efficient process. Once generated, Ang II rapidly binds to nearby AT₁ receptors, without leaking into the extracellular space. Aog = angiotensinogen.

Sequestration of circulating (pro)renin: diffusion or a receptor-mediated process?

The cardiac renin levels *per se* are too high to be explained based upon the amount of (renin- and prorenin-containing) blood plasma in the heart.^{14,15} Thus, circulating renin and prorenin either diffuse into the interstitial space¹⁹ and/or bind to (pro)renin receptors. In support of the latter, part of cardiac renin is membrane-associated.^{14,20} Moreover, isolated perfused hearts of rats transgenic for human angiotensinogen release Ang I during renin perfusion and this release continues after stopping the renin perfusion.²¹ These data support the idea that circulating renin binds to a cardiac renin-binding protein/receptor, and that bound renin is catalytically active.

The idea of renin binding is not new. In fact, evidence for renin binding was already obtained 20 years ago, when it was observed that vascular renin disappeared more slowly than circulating renin following a bilateral nephrectomy.²²

(Pro)renin receptors

Currently, two (pro)renin receptors have been identified.^{23,24} The mannose-6-phosphate (M6P) receptor binds renin and prorenin with high affinity in cardiomyocytes,²³ fibroblasts,²³ and endothelial cells.²⁵ This receptor is identical to the insulin-like growth factor II (IGFII) receptor, and as such it contains binding domains for both IGFII and phosphomannosylated (M6P-containing) proteins like renin and prorenin. Following binding, renin and prorenin are rapidly internalised, and internalised prorenin is proteolytically cleaved to renin.²⁵ (Pro)renin binding to M6P/IGFII receptors did not result in angiotensin generation,^{25,26} and (prorenin-derived) intracellular renin was found to be degraded slowly.²⁵ Thus, M6P/IGFII receptors most likely serve as clearance receptors for both renin and prorenin.

Nguyen *et al.* and Sealey *et al.*, using radiolabelled (pro)renin, demonstrated high-affinity renin binding sites/receptors in human

mesangial cells and in membranes prepared from rat tissues, respectively.^{27,28} Cloning revealed that the receptor is a 350-amino-acid protein with a single transmembrane domain.²⁴

The cloned renin receptor was found to bind prorenin equally well, and receptor-bound prorenin became enzymatically active in a non-proteolytic manner. These data support angiotensin generation on the cell surface (Figure 2).²⁶

Based on experiments with a series of antibodies directed against various parts of the prosegment, Suzuki *et al.*⁷ recently proposed that human prorenin has so-called 'gate' and 'handle' regions for its non-proteolytic activation. According to this concept, the handle region interacts with a putative receptor, which then leads to dissociation of the gate region from the renin molecule. Since this gate region is crucial for refolding and the maintenance of the inactive state, dissociation allows prorenin to display enzymatic activity. In a subsequent *in vivo* study, these investigators applied a decoy peptide corresponding to the handle region to block non-proteolytic prorenin activation.²⁹ This peptide reduced the renal content of Ang I and II and fully prevented the development of diabetic nephropathy in streptozotocin-induced diabetic rats. Thus, these data are the first to confirm that endogenous prorenin contributes to tissue Ang I and II generation in diabetic animals via a mechanism involving binding of its handle region to a receptor. It is tempting to speculate that this receptor is the above-mentioned (pro)renin receptor, but this remains to be proven.

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